

LOW LEVEL OF TRANSMITTED HIV DRUG RESISTANCE AT TWO HIV CARE CENTRES IN GHANA: A THRESHOLD SURVEY

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SUMMARY

Background: As access to antiretroviral therapy (ART) increases, the emergence and transmission of HIV drug resistant strains becomes a major problem. The World Health Organization (WHO) therefore recommends an initial minimum-resource method to signal when transmitted HIV drug resistance (HIVDR) requires action.

Objective: This survey sought to generate information on the presence of HIV drug-resistant strains in the locality where Ghana's ART for HIV was first introduced.

Methods: The Ghana HIVDR threshold survey (TS) was conducted and analyzed according to WHO strategy for surveillance of HIVDR in the Eastern Region of Ghana. Sixty (60) plasma specimens were collected from 2007 to 2009 by an unlinked anonymous method from HIV seropositive pregnant women, aged between 15 to 24 years, who were with their first pregnancy and ART naive. Genotyping was done as follows; Ribonucleic acid (RNA) was extracted from the samples and the protease (PR) and reverse transcriptase (RT) genes amplified and sequenced. The sequences were then analyzed for HIV drug resistance mutations using Stanford University HIV Drug Resistance Database.

Results: Only two individuals were found with major HIVDR mutations: one each in the PR and RT genes. Thus the level of HIVDR in the study population in 2009 was classified as low (< 5%).

Conclusion: As at February 2009, transmitted drug resistance was not a serious problem in the Eastern Region of Ghana. However, it is important to continue monitoring tHIVDR in order to understand the dynamics of the evolution of HIV drug resistance in the country.

INTRODUCTION

Combination antiretroviral therapy (ART) is known to improve treatment in HIV infected persons but the emergence of drug resistant viral strains is an obstacle in the effective management of HIV infection and AIDS.

Resistant strains may develop through selective pressure during ART or from spontaneously generated polymorphisms in rare cases. However, drug naïve persons could also be infected with drug-resistant strains.¹ Transmitted HIV drug resistance (tHIVDR) is therefore a major concern as it increases the risk of virologic failure.¹ This is particularly relevant in resource limited settings where treatment options are limited. Persons with tHIVDR commence therapy with a lower genetic barrier to resistance, higher risk of virologic failure and a higher risk of developing resistance to drugs in the regimen that they were originally susceptible to.²⁻⁵ Efforts to improve treatment outcomes and reduce transmission of HIV through the optimal delivery of ART and HIV prevention programmes must be accompanied by on going evaluations of HIV drug resistance.⁶

An essential element in the evaluation of the HIV situation is the population-based surveillance of tHIVDR in recently infected individuals.⁶ This will provide information to public health bodies to design educative and prevention programs and to inform the rational use of antiretroviral drugs by treatment programs, clinicians and policy makers. The prevalence of tHIVDR will probably remain low for several years in most countries where ART is being rapidly scaled up. The World Health Organization (WHO) therefore recommends an initial minimum-resource method to signal when transmitted HIVDR requires action.⁷

ART was introduced to Ghana in 2003. The program has been rapidly scaled up with an estimated 42,000 patients on ART in 141 care centres at the end of 2010.⁸ Ghana does not have adequate resources to monitor all persons on ART with viral load and drug resistance testing. Thus there was a need for population-based surveillance of HIVDR to evaluate the ART program to improve the outcome of the national treatment program.

The objective of this first ever threshold survey in Ghana was to generate information on active transmission of HIV drug-resistant strains in drug-naïve persons in the country.

METHODS

The Ghana HIVDR threshold survey was conducted and analyzed based on the World Health Organization (WHO) strategy for surveillance of transmitted HIVDR in resource limited settings. The details of the WHO strategy are previously discussed.⁶ Antenatal clients, with their first pregnancy, were used as proxy group of persons with recent HIV infections. The study protocol was reviewed and approved by the Ghana Health Services Ethical Review Committee.

Study area and population

Two ART sites in the Eastern Region of Ghana were used in this survey. The sites, located at the Atua Government Hospital and St. Martins de Porres Hospital, Agomanya, were selected because they were the pilot sites for the introduction of ART in 2003. These health facilities are national HIV sentinel survey sites and the HIV prevalence in the district indicated a reasonable sampling time. Pregnant women who were HIV-1 seropositive but ART-naïve, aged between 15 to 24 years, with their first pregnancy were enrolled into the study after obtaining their informed consent.

Sample collection and processing

The unlinked anonymous sampling method was used to collect blood samples from 60 eligible antenatal clinic clients who accessed Prevention of Mother-to-Child Transmission (PMTCT) of HIV services at the two sites from October 2007 to February 2009. Blood samples were processed into plasma at the collection point. The plasma was divided into aliquots of 0.5ml each and transported to the Public Health and Reference Laboratory (PHRL) in Accra in cold boxes. Specimens were accompanied by a form with sample identification number, date of blood collection and the number of vials attached. The PHRL conducted serology HIV testing by the Inno-lia HIV I/II (Innogenetics, Belgium) to determine the type of HIV infection. Three aliquots of specimens were then transported to the Noguchi Memorial Institute for Medical Research (NMIMR) and stored at -80°C until genotypic analysis was performed.

Genotyping

Viral ribonucleic acid (RNA) was extracted from 140 µl of plasma using the QIAamp Viral RNA Kit (QIAGEN, GmbH, Germany) according to the manufacturer's protocol. The viral load of the samples was estimated by an in-house real-time PCR assay using Taqman RT-PCR reagents (Applied Biosystems, Fos-

ter City, CA, USA) in an ABI 7300 Real Time PCR System. The protease (PR) and reverse transcriptase (RT) -coding genes were amplified in separate reactions using gene-specific primers as previously published.¹¹ The one-step RT-PCR kit (QIAGEN, GmbH, Germany) was used for the RT-PCR step [50°C 30min, 95° 15min, 40 cycles of (94°C 30sec, 55°C 1min, 72°C 90s), 72°C 7min] and the AmpliTaq Gold PCR Master Mix Kit (Applied Biosystems, Foster City, CA, USA) was used in a nested PCR [94° 2min, 40 cycles of (94°C 30sec, 56°C 1min, 72°C 1min), 72°C 7min]. The positive PCR products were purified using QIAquick PCR purification kit (QIAGEN, GmbH, Germany) and sequenced. All PCR reactions were performed in the GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA). The Big Dye Terminator cycle sequencing kit vs 3.1 (Applied Biosystems) was used. The cycle conditions were 94°C for 2mins followed by 25 cycles of 94°C for 30sec, 50°C for 15sec and 60°C 4mins. Sequence analysis was done in an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequence data was initially viewed and edited using DNA Sequencing Analysis Software 5.1 (Applied Biosystems Foster City, CA, USA). The sequences were then aligned using BioEdit software (Ibis Biosciences, Carlsbad, CA) and FASTA- formatted. The FASTA-formatted sequences were then submitted online to the Stanford University HIV Drug Resistance Database to generate mutation list, HIVDR and subtype information. The resulting mutation list was compared with the WHO mutation list for HIVDR surveillance.^{9,10}

Drug Resistance Mutation Analysis

HIVDR prevalence was categorized using the WHO TS binomial sequential sampling method.⁷ The details of the WHO protocol of determining HIVDR prevalence for threshold surveys was previously published.⁷ The specimens were listed in order of date of blood draw from the older to the newer specimens and the genotype results were examined. The "running total" of specimens with major HIVDR mutations was recorded and compared to the WHO recommendations. A classification of HIVDR prevalence was made based on the running total of specimens with major HIVDR mutations. A predetermined lower limit (LL) and upper limit (UL) were used for determining the prevalence. The prevalence of HIVDR was classified as $\leq 5\%$ if the total number of specimens with mutations was less than the LL and as $\geq 15\%$ if the running total of specimens with mutations was higher than the UL. If a total of 47 specimens were genotyped and the running total of specimens with major HIVDR mutations was neither less than the LL nor greater than the UL, prevalence was classified as in the range $>5\%$ to $<15\%$.

External Quality Control

One aliquot of plasma (0.5ml), for each of the 60 samples, was shipped on dry ice from the NMIMR, Ghana to the AIDS Virus Research Unit of National Institute for Communicable Diseases (NICD), Johannesburg, South Africa for quality assurance purposes. The samples were re-analyzed using an in-house (WHO-accredited) HIV genotyping protocol certified by the Virology Quality Assurance Program.

RESULTS

Of the 60 specimens analyzed, 53 were successfully sequenced for the RT gene. Seven samples could not be amplified. The first 47 samples were analyzed in line with the WHO recommendations for classifying the prevalence of transmitted HIVDR.⁷ One specimen (AGDR37) was found to have the mutations M184V

and Y181C as shown in Table 1. These are major HIVDR mutations as described on the WHO surveillance list.^{9, 10} Genotyping results from NICD corroborated the NMIMR analyses. The same mutations (M184V and Y181C) were found in the RT gene of sample AGDR37 from the independent analyses done in both institutions. Forty-six samples were successfully sequenced in the PR gene. Out of these, one sample had the mutation M46L as shown in Table 2. This is a major mutation that was recently added to the surveillance list for tHIVDR.⁹ The Stanford Database also classified most of the specimens as subtype CRF02_AG (74%) with 26% being subtypes A, G, K or CRF01_AE. Fourteen of the samples had viral loads below the detection limit of the assay (400 copies/ml) and those with detectable viral loads ranged from 503 to 315, 063 copies/ml.

Table 1 Major and minor HIVDR mutations found in the RT gene in specimens collected from HIV-1 seropositive ART-naïve pregnant women in the Eastern Region of Ghana (2007-2009)

Sample ID	Major DR Mutation	Minor DR mutation
AGDR4	NONE	E138Q
AGDR12	NONE	K101Q
AGDR35	NONE	K103R
AGDR37	M184V, Y181C	NONE
AGDR41	NONE	A98G

A98G is a minor mutation that reduces Nevirapine susceptibility by 2 to 3-fold. It is selected by Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs). K101Q is weakly associated with NNRTI therapy and minimally reduces susceptibility to each of the NNRTIs.

K103R occurs in about 1%-2% of untreated persons and by itself has no effect on NNRTI susceptibility.

E138Q is weakly associated with NNRTI therapy. It may contribute to decreased NNRTI susceptibility in certain genetic contexts.

Table 2 Major and minor HIVDR mutations found in the PR gene in specimens collected from HIV-1 seropositive ART-naïve pregnant women in the Eastern Region of Ghana (2007-2009)

Sample ID	Major DR Mutation	Minor DR mutation
AGDR5	NONE	V11I
ATDR62	NONE	L10I
ATDR63	NONE	L10I
AGDR9	NONE	N83D
AGDR12	NONE	L10I
ATDR14	NONE	L10V
AGDR21	NONE	V11I
AGDR24	NONE	I50F
ATDR23	NONE	D30G
AGDR32	NONE	L10I
AGDR34	M46L	I84L

D30G is a highly unusual mutation at this position

L10I/V is known to occur in 5-10% of untreated persons.

M46L decreases susceptibility to the Protease Inhibitors (PIs) but only when it is present with other relevant mutations.

I84L is a highly unusual mutation at this position and it is not yet implicated in resistance to PIs.

N83D is a non-polymorphic mutation that occurs more commonly in heavily treated patients. It has been associated with decreased virological response to TPV/r. Its effect on other PIs is not known

I50F is a highly unusual mutation at this position.

DISCUSSION

The list of mutations used for the interpretation of results was those published in the Surveillance Drug Resistance Mutation (SDRM) worksheet.⁹ After analyzing the required numbers of sample, as prescribed by the WHO,⁷ one sample each showed resistance mutations in the RT and PR genes. Minor mutations were also seen in the samples analyzed as shown in Table 1 and Table 2. The minor mutations observed in the RT gene included E138Q, K101Q, K103R and A98G while those observed in the PR gene included L101I/V, V11I, N83D, I50F, D30G and I84L. These do not confer drug resistance by themselves and were not listed on the SDRM. The level of tHIVDR in the study population was therefore classified as low (< 5%). These specimens were collected from the pilot sites where ART was first introduced in Ghana in 2003 and so represent a population that has the longest experience with ART in Ghana.

The low level of tHIVDR observed in this population is an indication that the 1st and 2nd line drug regimens should be beneficial to patients who are yet to start treatment. Similar studies in Ethiopia and Malawi have reported low levels of tHIVDR.^{12, 13} Another study in treatment-naïve HIV infected individuals in Ghana, previously reported the absence of major drug resistance mutations in either the RT or the PR genes.¹⁴ However, one study done in East and Southern Africa described increasing trends of tHIVDR over a 4 year period and another from four countries in Central Africa reported low (<5%) to medium (5%-15%) prevalence of tHIVDR.^{1,15} The low tHIVDR level observed in this study calls for continuous monitoring of tHIVDR. This study should be repeated after 2 years at the same sites and later extended to other sites for a continuous assessment of tHIVDR in Ghana

CONCLUSION

The level of tHIVDR in the Eastern Region of Ghana in 2009 could be classified as low (\leq 5%). However, it is important to continue monitoring tHIVDR in order to understand the dynamics of the evolution of HIV drug resistance in the country. An HIV genotyping laboratory has been established at the NMIMR to enhance the local capacity to conduct HIVDR testing in support of the national ART program of the Ghana Health Service.

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